F16.

NUCLEIC ACID AND PREDICTED PROTEIN SEQUENCE OF DZ2

09	120	180	240	300	360
Neol SCAGAATCGAAGAAGAAATCCATGGGAGATATCGAGAAATAAAGAA 60 M A T K S M G D I E K I K K	CCTTATAATTCATGAGAAGAT 120 L I H E K I	UZ2FL→ GGATTGGGGGTATTTCACAGACAGCGAATAACGGTGAGGAGGCAGTAATCAT 180 I G G I S Q T A N N G E E A V I I	GGATAAAGAAATGCCCGAGAG 240 D K E M P E R	TTTCGACAACTAAGAAAATGGAAGTGAAGTCAATGATTGTTGG 300 / S T T K K L R E M E V K S M I V G	CACTGGCTGACAATGAAGAGGGCGCAGGGCTTTCATGGAAGCTGGACTTAA 360
Ncol ATCCATGGG	TCCACTAAAC P L N	AGCGAATAAC A N N	TATCCTAATC I L M	AGAAATGGAA E M E	GCGCAGGGCT R R A
GGCACGAGCAGATCGAAGATGGCAACAAA	GAAACTAAACGTGTTGATGATGATCCACTAAACCTTATAATTCATGAGAAGAT 120 K L N V L I V D D D P L N L I I H E K I	↓ ATCAAAGCGATTGGGGGTATTTCACAGAC I K A I G G I S Q T	181 <u>ccaccgtgacggctcatcttttgaccttatcctaatggataaagaaatgcccgagag</u> 240 H R D G G S S F D L I L M D K E M P E R	GATGGTGTTTCGACAACTAAGAAGCTAAG D G V S T T K K L R	GTGACTTCACTGGCTGACAATGAAGAGGA V T S L A D N E E E
1 GGCP	61 GAAA K	121 CATCAAAG I K A	181 <u>c</u> cac	241 GGATGGTG D G V	301 GGTGACTT V T S

1/27

420 CCATTGCTTGGCAAAACCGTTAACCAAGGACAAGATCATCCCTCTCATTAACCAACTCAT M I O 361

480 GGATGCTTGATGGATATATTTTATATTATGGAAACACACATAATAACGTCTAAGTGTG ← DZ2RL 421

009 540 TATGTATGCATAGATACTTGCATGTGTGTTTTAGAATTTAGGGTTCTTTATCGTCCGT 541 GATATATAATCATGTAAGTTGTTGCTTTAAGCTTATAAAATATAAGGGTTTCCT HindIII481

2/27

601 CTACC

The primer sites for DZ2FL and DZ2RL are underlined, as are the recognition sequences for Ncol and HindIII restriction enzymes. Shown in bold are the conserved amino acid residues required phosphorylation. The extent of the original PCR product isolated by differential display is shown by ↓

FIG. 1 CONTI

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Alignment of the predicted protein sequence of DZ2 with those of bacterial response regulator proteins.

DZ2 OMPR PHOB NTRC SPOOF CHEY ETR	1 MATKSMGDIE	.MQENYKILVMARRILVMQRGIVWVMMNEKILI MADKELKFLV	VDDDPLNLII VDDDMRLRAL VEDEAPIREM VDDDSSIRWV VDDQYGIRIL VDDFSTMRRI MDENGVSRMV	HEKIIKAIG. LERYLTEQGF VCFVLEQNGF LERALAGAGL LNEVFNKEGY VRNLLKELGF TKGLLVHLGC	50 GISQTANNGE .QVRSVANAE .QPVEAEDYD .TCTTFENGN .QTFQAANGL NNVEEAEIGV EVTTVSSNEE
DZ2 OMPR PHOB NTRC SPOOF CHEY ETR	QMDRLLTR SAVNQLNE EVLAALAS QALDIVTK DALNKLQACLRVVS	KTPDVLLSDI ERPDLVLLDM	MLPGEDGLSI MLPGGSGIQF RMPGMDGLAL KIPGMDGIEI NMPNMDGLEL	TKKLREMEVK CRRLRSQS IKHLKRESMT LKQIKQRH LKRMKVID LKTIRADGAM ALRI	NPMPIIMVTA RDIPVVMLTA PMLPVIIMTA ENIRVIIMTA SALPVLMVTA
DZ2 OMPR PHOB NTRC SPOOF CHEY ETR	LADNEERRA KGEEVDRIVG RGEEEDRVRG HSDLDAAVSA YGELDMIQES EAKKENIIAA GNTDKSTKEK	.LEIGADDYI .LETGADDYI .YQQGAFDYL .KELGALTHF .AQAGASGYV	TKPFSPKELV PKPFDIDEAV	ARIRAVLRRQ ARIKAVMRRI ALVERAISHY DAVKKYLPLK EKLNKIFEKL	SPM QEQQQPRNIE SN
DZ2 OMPR PHOB NTRC SPOOF CHEY ETR	51 VNGP 	PhoB = E. NtrC = S. SpoOF = B. CheY = E. ETR = A.	coli (Involved in typhimurium (In subtilis (Involve coli (Involved in	gene encoding an	ation)

The predicted protein sequence of DZ2 is shown in bold as are the conserved amino acid residues required for phosphorylation of the protein

FIG.2



Expression analysis of DZ2 in various plant organs using Northerns

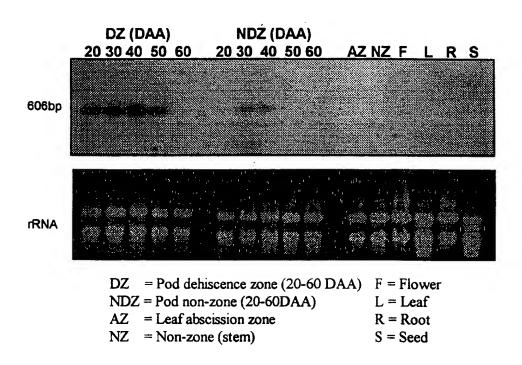


FIG. 3

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Comparison of bacterial two-component regulatory systems with DZ2

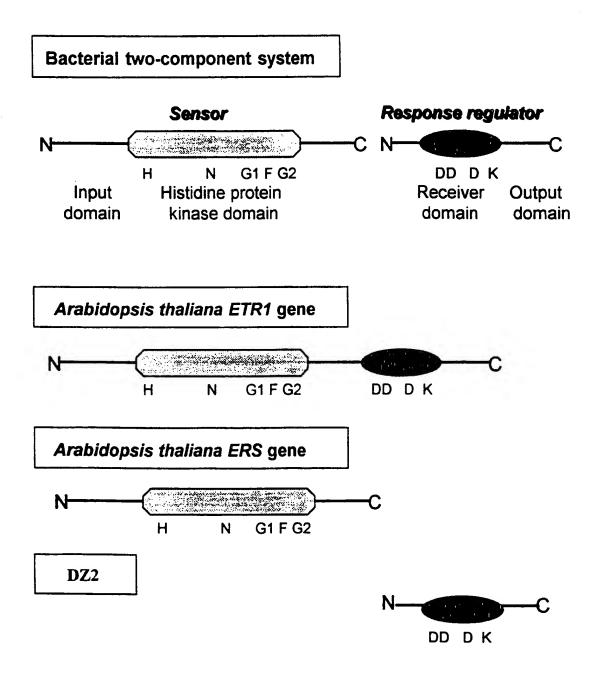


FIG. 4

F16. 5

TATATAAATACGGTTTAACAGATATGTTCTGGTTATAAATGTAATTCNATGTGCCNNTCAANTYTTATTTTNATTNGT	78	
TNTACTAGGGACATTAGTTTTAACNTTTTATATATCATGTAACAAAAAAAAAA	156	
GCAATTATTCTTATAGTGTTTTTCTTTTTCCAGAAATTTGACGACAACCTAAACTAAAACAATTTAATTTGACGTTAGTT	234	
AAGTAATTTATATAGATGATAAATTGAGCAAGCACATTACGAACTGCGGATCAAGGAGAGTCACAATTTÄATTCTTA GGC <u>TCTAGA</u> CGAACTGCGGAGCAAGG -> DZ2BGENF XDaI	312	
CGTTATACACAAAATTATCTAAATACTATATATATATACAGCTGCATGCTACGATAATGATCAAATGTTTATGTACTT	6/27	ב ו ייי
TTCAGCGAAAATTCTTGTCGCCATACATTACTGTGTTAATGAATCATTAAATATGTGAAGGAGGAAAAAGAGTACAAAA	468	
GGAGTTTTGTTGAGGCATTTCGCAGACACTGAAATGTGAATAATAATAAGGAATTGCCGAATTGATTTCTAGTTGGT	546	
GAAGTGGGTGAAAATTGTATGTCCATTGCTTATAAACTATAAAATATATAT	624	
TINIGATAGACCCTAGCTAAAATTTTTAAAAATTATACATTCATT	702	
TAAAATTGTTTAAGAAACCATTACAAACTCAGCTTGTGGACTCTGAGAGAAACTAAGAGCTAGACATACGGTTAGTAG	780	
TGTAGCCGCATTTTTTATGCTTAATTTTGCTTAAGCATGACTTCTATGCTCCTTGATGATGATATTTTAATATCCTAG	858	
GACATATGGATTTGATAAAGATCTTATCAACCTTTCAACAAGACCATTAGCTCAACAAACA	936	

ATTAATATATTTTTCACTTTTTGTTTTATGTATTCTTAGTATTTTGTTCACCATATTGACCGATTGGTGTCATATTA

GGTGTGAAAATATGCCTCTTTCACTTTTTTCAACTATAAATTTCGATGATGTATCTACGTTCTTAACACAATTCAC

CGCATGCATTAGAACACTTGTATGTTAATTTTCCATAATGTTTTTGCATAAACATTCTTCGTTTTAATTAGCTTCTTTTT

1092

1248

1326

1404

AACCAAG

DZ2BGENR

CTTCGAAGAAAAAAACCGACCATGGCAG

NCOL

1638

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TCAGTAGAAGTGAAGAAGAACTTAACGTGTTGATCGTTGATGATGATACAGTAATTTCGTAAACTTCACGAGAATATC Z 回 I H × ĸ Н > H Ω Ω Ω > > z DZ2BFL ы × × TCAGTAGAAGTG ->

1716 ATCAAATCGATGGAATTTCACAGACGGCTAAGAACGGTGAGGAGGCAGTGAACATCCACCGCGACGGCAATGCA Z Ö æ E O z H S O

FIG. 5 contro

The first than the first first

A) Dz2B cDNA segence (top)

8 / 27

9/27

GAGCGCAGGGCTTTCATGGAAGCTGGACTTAACCATTGCTTGGCAAAACCGTTAACCAAGGACAAGATCATCCCTCTCATTA

ACAATCTCATGGATGCTTGATGGATGAATTGTCGCCACTACATATCTACATTATACAAATATGAAAAACACATATAAT ACCAACTCATGGATGCTTGATGGATATATATTTTATATTATG-

••••••••••••••

--GAAACACACATAAT

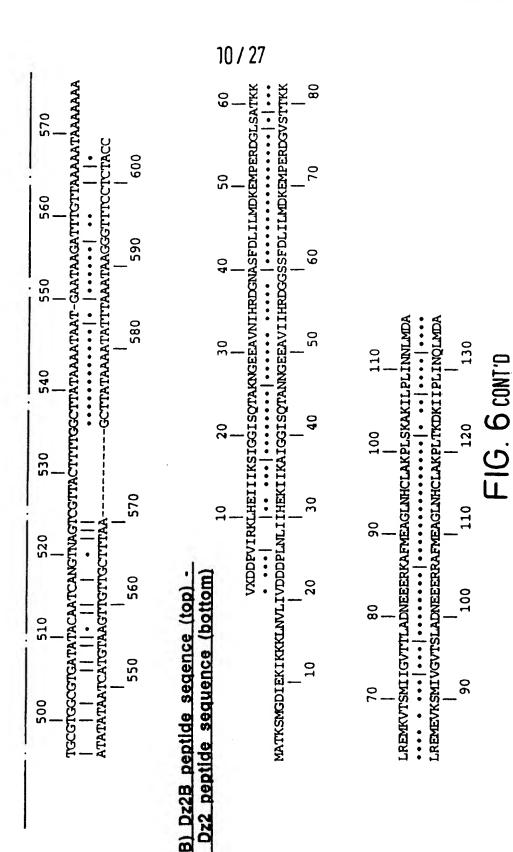
AACGICATACACCIGIGIG--IGIAIGCATAGATAICTATCCGCAIGIGIGITITITAGGGGTIGTTAIGTTIGATITITIAITIG

AACGICCIA

• • • • • •

--AGTGTGTATGTATGCATAGATACTTGCAT--GTGTGTTTTTAGAATTTTAGGGTTCTTTTATCGTCCGTG

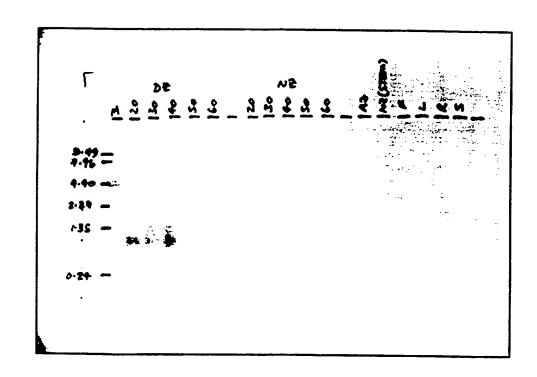
FIG. 6 contro



11/27

FIG. 7

NORTHERN ANALYSIS OF EXPRESSION OF DZ2B IN PODS AND OTHER TISSUES



DZ = POD DEHISCIENCE ZONE (20 - 06 daa)

NZ = POD NON-ZONE

AZ = ABSCISSION ZONE

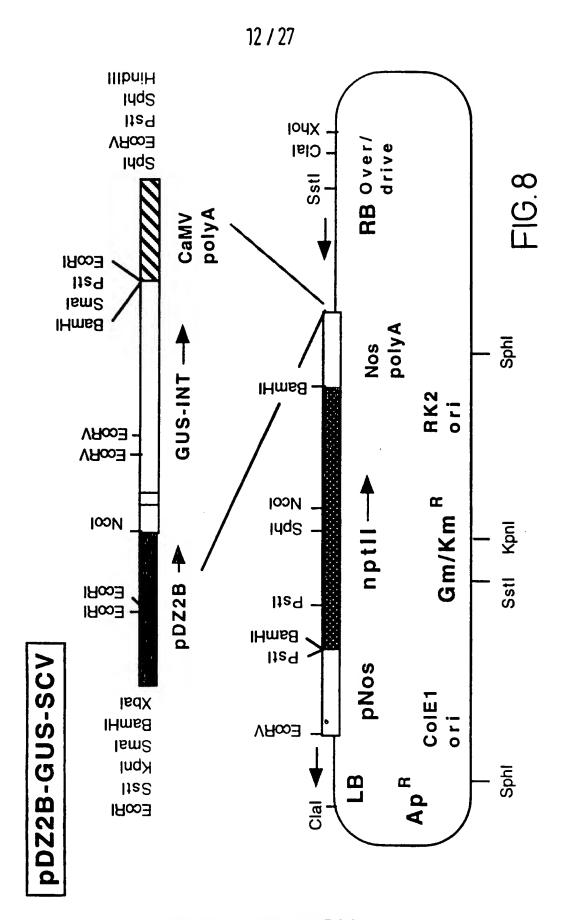
F = FLOWER

L = LEAF

R = ROOT

S = SEED

TENSOR OF DEPOSIT



F16.9

78 **ATATATGTGATACAGATACATCTATATACAAATTAAACACGAAACCATACATGCACGGTGTGATCACACACGCACACA**

156

234 TCTTGAGAGAAAAGAAATCGAAGATGGCAACAAAAATCCACGGGAGGTACCGAGAAAACCAAGTCGATAGAAGTGAAGA S × ¥ Ы E Ö Ö ഗ × Ø

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312 <- CGATCG AGAAACTAATCAACGTGTTGATCGTCGATGATCCATTAAACCGTAGACTCCACGAGATGATCATCAAAACGATCG 又 Н Σ 回 I H 公 Z Ω Ω Ω > Ц

GW1 <- CGAATAACGGTGAGGAGGCATTAATCA</p>

AT3GW2

GAGGAATTTCTCAGACTGCAA

390 GAGGAATTICICAGACTGCAAAGAATGGCGAAGAGGCNGTGATCCTCCACCGTGACGGCGAAGCATCTTTCGACCTTA

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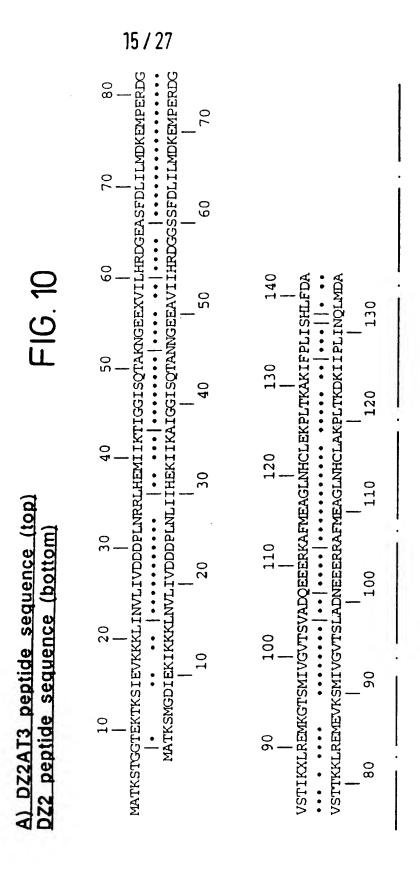
TITCTCATATCTATGTTTGATTTTTTTTTTTTTGTCGAGGTAAAATCATGCAAGTCATTTCTTTTGGCTAATAAAA

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AAG G	ICA N	IGA	(
K K	Š T	KGA.	Š
AA1 M	9 13 13	ATC	6
AGA E	AGC A		ç
GATGGAGTTTCGACAATTAAGANGCTAAGAGAAATGAAAGGGACGTCAA D G V S T I K X L R E M K G T S M	CAAGAAGAAGCGTAAGGCTTTTATGGAAGCTGGGCTCAACCATTGCT Q E E E R K A F M E A G L N H C L	<u>ک</u> ور	Ì
CT2	ATC M	TTCCCGCTCATTAGCCACCTCTTCGATGC	F
ANG	ITTT	TTC	*
AAG.	J.C.I.	Ď N	6
ZII.	AGC	.ACC	Ş
CAA	GTA K	OO H	į
GAP T	AGO R	TTA S	É
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TICTAATGGATAAGGAAATGCGTGAGAGTTTCGACAATTAAGANGCTAAGAGAAATGAAAGGGACGTCAA L M D K E M P E R D G V S T I K X L R E M K G T S M	TGATCGTTGGGTAACGTAGCTGACCAAGAAGAGCGTAAGGCTTTTATGGAAGCTGGGCTCAACCATTGCT I V G V T S V A D Q E E E R K A F M E A G L N H C L	TGGAAAAACCCTTAACCAAGATCTTCCCGCTCATTAGCCACCTCTTCGATGCATGAAGGCTCATT E K P L T K A K I F P L I S H L F D A •	7
CHA I	ATC	SAA	A A TICH A THE THE THE TANK A MATCH A THICK A A A
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FIG. 9 contro

TATTAAAATAAGGTTTTCTCAAAAAAAAAAAAAAA



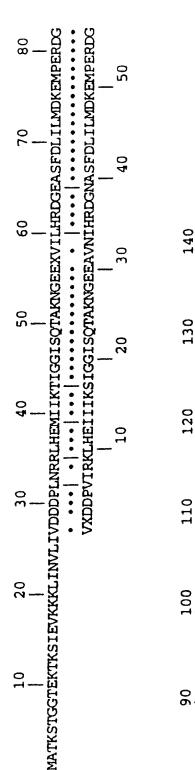


FIG. 10 cont'd

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VSTIKXLREMKGTSMIVGVTSVADQEEERKAFMEAGLNHCLEKPLTKAKIFPLISHLFDA

LSATKKLREMKVTSMI IGVTTLADNEEERKAFMEAGLNHCLAKPLSKAKILPLINNLMDA

B) DZ2AT3 peptide sequence (top) DZ2B peptide sequence (bottom)

FIG. 11

			17,	/ 27					
78	156	234	312	390	468	546	624	702	
GTAATGCGACTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGTCCTCATTCGTATTGGGCCCAATGGGCTACT CACTAGTAGGCCACGCGTGGTCG -> ATDZ2F SpeI	AAAACAGTITCACGATTGITTTTTTTTTTTTTTTTTTTTT	TAAAAAATTTCACGATAGATTGAATTTTTGAATTTCGAGTTAAAATATCTTCAAATTACCTCACATTTACAAAAA	GGTAGAACTGTTGAAAAACTAATGCTCTATAAAACACTAGACAATAACAAAATACGTAATGCGTAAAGAACCTAAATT	ATGATTTTATTTATCTTTCTTCCTTTTTTCCGTGAGTATAAGCCATTTTTCATAGTAAAGCATTACGAATACGACATTG	AACACTACTGACATATAAAGTAGTAGATTTTGATGGGTTAACTTGTATGCTTAATTTGCTTAAGCATGAACTTCAATG	CTTTTATAAAAGTACTTCATGAGAATATTCCTCGTTCTATACTAGCAGAAGGGTTCGATAGTGATTTTACAACCGTTC	AACAAAACCTTTAAACCCAAAAAACCAAAGAATGAAAGTATCTAAACTTGATTATACATTTCTTGTCTAAATTATCAA	ATAACATACTCTCTTTTTGTTTATAAACGATATGAAAGAAA	

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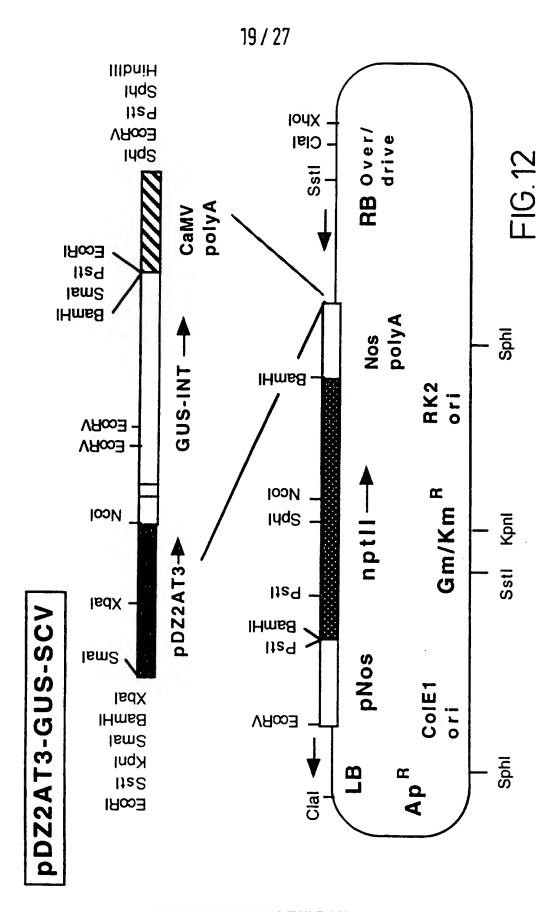
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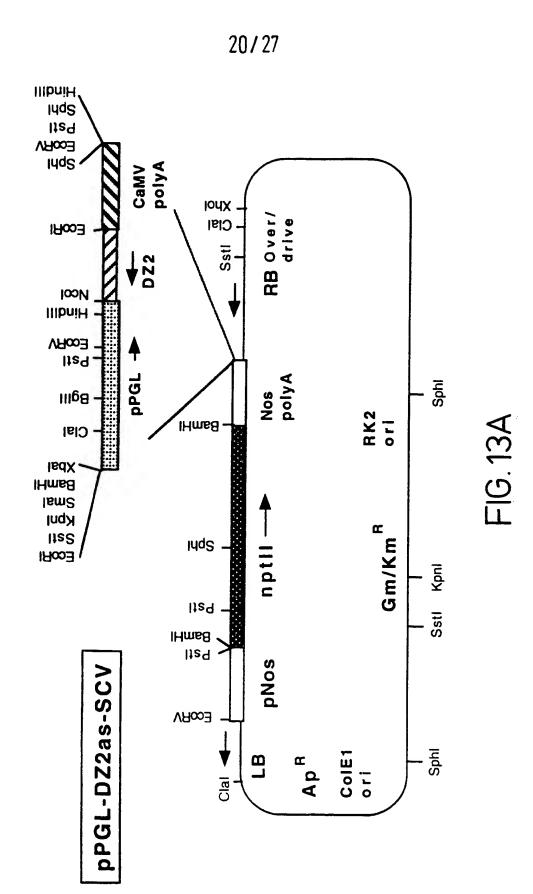
18 / 27	TATGTAATATATGTGATACAGATACATNTATATACAAATTAAACCGGAAACCATACATGCAGGTGTGATCACACGG 1092 CACACACACATAGAAACGAAAATTTCTATACATTTAATTTCATTTTTAACTTTCTTT
1170	• •
32	109
m	1013
	936
	828
	780

FIG. 11 cont.

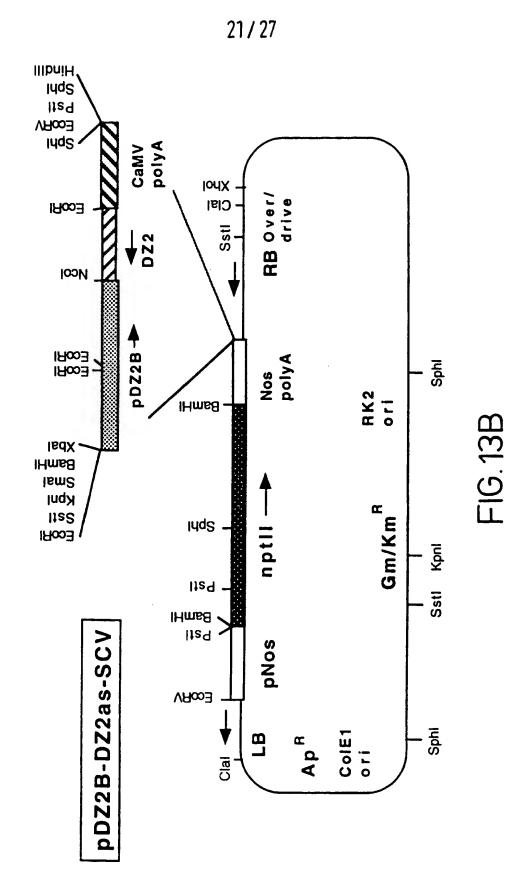
TGAAGAAGAAACTAATCAACGTGTTGATCGTCGATGATGATCCATTAAACCGTAGACTCCACGAGTGTCATCAAAA

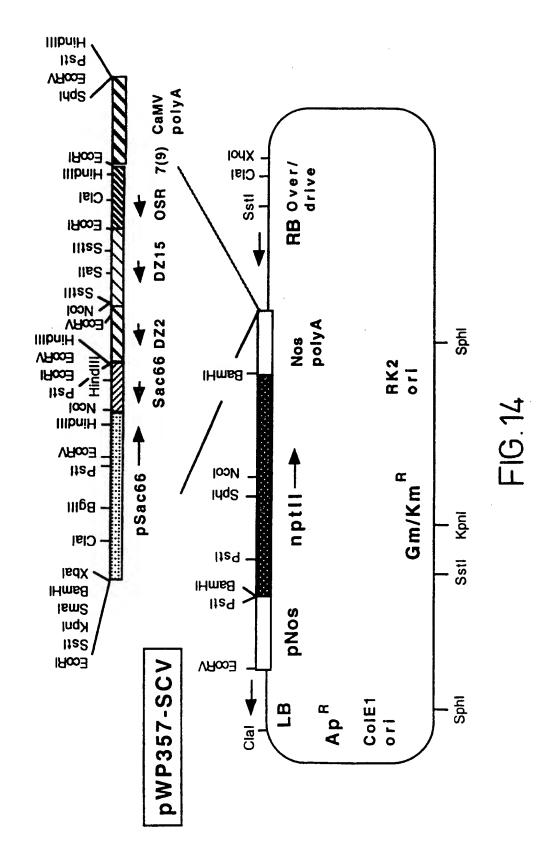
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GGCATCACGAGGGTACCCGTAAATCCCACCATACAACAAGTTCTGTGAAAGTCTCCCAA

AAACTGCAAAGAGTCTCATATTAGTTCTTACTCTCAGAAATAAAACACAGTGTTTCTGAA AAGATTAGCGTTTCAAACCCCGAAATGGCCCGTTGTCATGGAAGTCTTGCTATTTTTTA ပ

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TGCGTTCTTTTGATGCTTGCTTGCCAAGCTTTGAGTAGCAACGTAGATGATGGATAT

GGTCATGAAGATGGAAGCTTCGAAACCGATAGTTTAATCAAGCTCAACAACGACGACGAC Z Ц S ㅁ S G

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360 GTTCTTACCTTGAAAAGCTCCGATAGACCCACTACCGAATCATCAACTGTTAGTGTTTCG S S

AACTTCGGAGCAAAAGGTGATGGAAAACCGATGATACTCAGGCTTTCAAGAAAGCATGG N F G A K G D G K T D D T Q A F K K A W

AAGAAGGCATGTTCAACAAATGGAGTGACTACTTTCTTGATTCCTAAAGGGAAGACTTAT ဌ Д ⊱ ဗ ပ

540 CTCCTTAAGTCTATTAGATTCAGAGGCCCATGCAAATCATTACGTAGCTTCCAGATCCTA ۵,

152 GGCACTTTATCAGCTTCTACAAACGATCGGATTACAGTAATGACAAGAACCACTGGCTT so. S 2 ×

GCGCCAACGGCTCTTACTCTACAACCTAAACAATTTGAATGTGAAGAATCTGAGAGTG A P T A L T L Y N L N L N V K N L R V AGAAATGCACAGATTCAGATTTCGATTGAGAATGCAACAGTGTTGATGTTAAGAAT
GTTAAGATCACTGCTCCTGGCGATAGTCCCAACACGGATGGTATTCATATCGTTGCTACT V K I T A P G D S P N T D G I H I V A T
AAAAACATTCGAATTCAGACATTGGGACAGGTGATGCATATCCATTGAG K N I R I S N S D I G T G D D C I S I E
GATGGATCCAAATGTTCAAATCAATGATTTAACTTGCGGCCCCGGTCATGGCATCAGC 1020 D G S Q N V Q I N D L T C G P G H G I S 292
ATTGGAAGCTTGGGGGATGACAATTCCAAAGCTTATGTATCGGGAATTAATGTGGATGGT 1080 I G S L G D D N S K A Y V S G I N V D G 312

FIG. 15 CONTO

GCTACGCTCTCTGAGACTGACATGGAGTAAGAATCAAGACTTACCAGGGAGGG	1140 332 1200 352 1260 372 1320
	1380 412 1440 432
	1500 433
GCCATCTATATCTACGTATTGATTCTCGATATATATAGAAACTAAGATTTAT	1560
GGGAATATACATACAATAGTTGAGATAATTGTTGTCTTGTATATGGTTCACTGAAGTTGA	1620
ttgcttgtccacgaataatgaataatgtcatttgtc FIG. 15conto	1657

aggtgaccgttgctgatggcaatgtgctggtcaagcgagaggtagacggtggcttggaga D G N V L V K R E V D G G cagttaaagtcaaattgccagctgtcattagcgccgacttgcggctcaatgagccgcggt V K V K L P A V I S A D L R L N E P R acgctactctgcccaatatcatgaaggccaagaagaagcccatcaaaaagctcacagccaATLPNIMKAKKPIKKL cagatgtcggtgtggacttggcgccacgtcaacaagtgttgagcgtagaagacccgccca D L A P R Q Q V L S E RQAGSIVPDVDTLITKLKE agggtcatttgtaatgcaatgtcaccaatacagttgttttagttcttacaaattcttcgt G H L * ${\tt gaggttttcagctgttaccaataatattttttcaaaatcgattttattttacttgtaatt}$

FIG. 16

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	ggtcgaaccataggtggaaagcttctttttttttgcttgacaaatctttggtt G R L L S L D K S S G S		gggtttcagtccatcaggagttctctatggtaaagctgaggttcaaatgaaacttg G $ m K$ A $ m E$ V O M $ m K$ L V G $ m K$ A $ m E$ V O M $ m K$ L V		actctgctggaacagtcacattctatcttaaatcaccgggaactacat		tcgatttcgagttcttgggaaacataagtggccatccctatactctccata \mathtt{D} \mathtt{F} \mathtt{E} \mathtt{F} \mathtt{L} \mathtt{G} \mathtt{N} \mathtt{I} \mathtt{S} \mathtt{G} \mathtt{H} \mathtt{P} \mathtt{Y} \mathtt{T} \mathtt{L} \mathtt{H} \mathtt{T}		acacacgaaggctctggagacaaagaacagcagttcatctatggttcgac T R R L W R Q R T A V S S M V R P
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